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Bioconversion of food waste to volatile fatty acids: Impact of microbial community, pH and retention time

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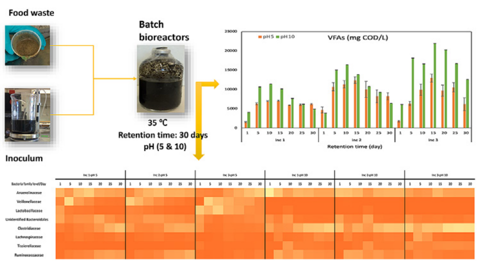
HIGHLIGHTS

- Shifting pH from 5 to 10, substantially increased the VFA production.
- Inoculum acclimated to food waste resulted in the highest VFA production.
- Acetic acid was the dominant product of alkaline condition.
- Acetic acid and propionic acid were the dominant products of acidic condition.
- *Clostridiaceae* highly correlated with acetic acid production in both pH conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Bio-based production of materials from waste streams is a pivotal aspect in a circular economy. This study aimed to investigate the influence of inoculum (three different sludge taken from anaerobic digestors), pH (5 & 10) and retention time on production of total volatile fatty acids (VFAs), VFA composition as well as the microbial community during anaerobic digestion of food waste. The highest VFA production was ~22000 ± 1036 mg COD/L and 12927 ± 1029 mg COD/L on day 15 using the inoculum acclimated to food waste at pH 10 and pH 5, respectively. Acetic acid was the dominant VFA in the batch reactors with initial alkaline conditions, whereas both propionic and acetic acids were the dominant products in the acidic condition. *Firmicutes*, *Chloroflexi* and *Bacteroidetes* had the highest relative abundance in the reactors. VFA generation was positively correlated to the relative abundance of *Firmicutes*.

1. Introduction

Resource recovery from waste streams is one of the pillars of transitioning into a circular economy and meeting the environmental sustainability agendas set by United Nations. The escalating global trend of waste generation is of major concern in the 21st

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century. Food waste (FW) is deemed as one of the main constituents of the municipal solid waste (Ren et al., 2018). Approximately, one third of the food generated globally is discarded as waste every year (Xu et al., 2018). Current prognosis for the EU indicates that 88 ± 14 Million tonnes (Mt) of food waste is produced along the supply chain, which is equivalent to 173 kg ± 27 kg per capita/year (Scherhaufer et al., 2018). These numbers are projected to increase due to rapid global urbanization and change of lifestyles.

The report of the European Market for bio-based chemicals stated that only 3% of the total chemical production in Europe is supplied by bio-based production methods (Spekreijse et al., 2019). Particularly, 0.3% of the platform chemicals such as volatile fatty acids (VFAs), ethanol, lactic acid etc. are produced through bio-based techniques (Spekreijse et al., 2019). Nevertheless, the demand for bio-based chemicals are increasing day by day (European Commission, 2019). It is estimated that the bio-based chemical need will reach 6134 kt/a with 2% CAGR in 2025 (Spekreijse et al., 2019). In other respects, an assessment by the European Commission (Commision Expert Group on Bio-based Products, 2017) indicated that bio-based products represent approximately EUR 57 billion in annual revenue and involve 300,000 jobs. According to forecasts, the bio-based share of all chemical sales will rise to 12.3% by 2015 and to 22% by 2020, with a compounded annual growth rate of close to 20%. The so called “Greening of society” reflected in a growing consumer preference for green products. For many companies, bio-based products are therefore seen as an investment for the future replacing e.g. chemicals of fossil origin.

Conventional waste treatment techniques such as landfilling impose detrimental environmental effects such as greenhouse gas and malodourous emissions. Another method is incineration which is not only expensive, but also energy intensive (Hobbs et al., 2018; Ren et al., 2018).

Anerobic digestion (AD) on the other hand, is a cost-effective alternative to traditional waste treatment methods. Particularly, as realization of next generation wastewater treatment plants opens new avenues in proper waste management as well as energy production and recovery of value-added materials from waste streams. Biogas generation has been the focus of AD processes until the recent years. However, increasing demand for high added value bioproducts is the driving force for shifting AD processes towards volatile fatty acids (VFAs) production (Kleerebezem et al., 2015). VFAs contain six or less carbon atoms and are the intermediary products of AD. They can be exploited as renewable carbon sources and building blocks in different industries (Atasoy et al., 2018) such as in bioplastics production (Khatami et al., 2020), biodiesel (Lee et al., 2014), biological nutrient removal processes (Strazzera et al., 2018) as well as electricity generation (Xu et al., 2018).

To date, VFAs are mostly obtained through chemical processes using petrochemicals as the raw material (Zacharof and Lovitt, 2013). Bio-based production of VFAs through anaerobic fermentation is more appealing as it is not only cost-effective, but also environmentally friendly (Capson-Tojo et al., 2016). Food waste is a suitable feedstock for AD processes due to its abundance, facile biodegradability, nutrient-rich nature as well as high energy and moisture content (Hobbs et al., 2018; Ye et al., 2018; Zhang et al., 2014). In spite of the advantages presented by biobased production of VFAs, their commercial production is yet limited (Kim et al., 2018). Separation of the products from the fermentation broth is considered as one of the main bottlenecks (Rebecchi et al., 2016). Although high recovery efficiencies have been reported from liquid-liquid extraction, adsorption and ion exchange techniques, their high costs and environmental impacts are still burdens (Atasoy et al., 2018; Strazzera et al., 2018). Meanwhile, application of membrane separation techniques are promising as they realize the concurrent recovery of the VFAs during their formation in the bioreactors (Trad et al., 2015).

Several studies have focused on optimizing the operational parameters to enhance VFA production through AD. For instance, temperature impacts the growth of microorganisms and excretion of the enzymes as well as the hydrolysis of the organic materials (Kim et al., 2003). Garcia-Aguirre et al. (2017) evaluated the influence of temperature on AD of seven different organic waste streams and obtained the highest VFA concentration of ~8000 mgCOD/L from fermentation of the organic fraction of municipal solid waste under mesophilic conditions at alkaline pH. Furthermore, Komemoto et al. (2009) discovered that under mesophilic temperatures (35 °C and 45 °C), anaerobic digestion of FW has higher solubilization rates compared to thermophilic (55 °C and 65 °C) conditions. Another critical parameter is pH as it regulates the activities of different microbes involved in AD (Ye et al., 2018). It is known that neutral pH, between 6.8 and 7.2 is the optimal range for the growth of methanogens (Liu et al., 2011). The influence of pH on acidogenic fermentation of FW was investigated by (Dahiya et al., 2015). Adjusting the initial pH at different values the highest VFA productivity was acquired under pH 10 (Dahiya et al., 2015). Inoculum also plays a crucial role in the performance of the AD processes due to their influence on fermentative pathways (De Gioannis et al., 2013) as well as product formation (Zhou et al., 2018). In another study, Li et al. (2018) demonstrated the significance of the genera *Clostridium*, *Ruminococcus* and *Prevotella* in VFA and hydrogen production from FW. Despite these studies, the impact of the microbial structure on the fermentative production of VFAs is still a grey area and further research is needed to increase the efficiency of VFA production from waste and to commercialize the bio-based VFA production.

In this view, the aim of the current study is to investigate the role and impact of bacterial community in conjunction with operational parameters as pH and retention time on VFA production. Three different sludge obtained from anaerobic digestors were used as inocula in batch acidogenic fermentation of FW. Structural changes of the microbial community under both acidic and alkaline conditions in short term lab scale reactors and their influence on the VFA production and composition with respect to time was studied in detail.

2. Materials and methods

2.1. Food waste and inoculum

The food waste was collected from Syvab wastewater treatment plant in south of Stockholm, Sweden. It contained household food waste and the faulty batches of breweries (when the end-product of the breweries does not pass the quality control and cannot be sent for sale) hygienized at 71 °C for 61 min in accordance with the Swedish law. No further pre-treatment was applied to the food waste after arrival to our laboratory as the hygenization can be considered as a thermal pre-treatment. The first inoculum originated from the full-scale mesophilic anaerobic digestion tanks of Henriksdal wastewater treatment plant, Stockholm, Sweden, treating a mixture of primary sludge (PS), waste activated sludge (WAS) and restaurant food waste (RFW). The second inoculum was obtained from full-scale mesophilic anaerobic digesters of Henriksdal wastewater treatment plant which treats PS, WAS and glycerol (G). The third inoculum was collected from the food waste digesters of the Scandinavian biogas facilities in south of Stockholm. Table 1 summarizes the characteristics of the substrate and the inocula with their respective acclimated waste streams. Prior to the experiments, all the inocula and food waste were stored at 4 °C.

Table 1
Substrate and inoculum characteristics.

	FW	Inoculum 1	Inoculum 2	Inoculum 3
		PS/WAS/RFW	PS/WAS/G	DFW
TS [mg/L]	146233	23166	23666	41800
VS [mg/L]	13266	15700	15800	30766
VFA [mg/L]	5739	_	_	_
pH	5.53	7.37	7.1	7.58
TCOD [mg/L]	215050	53200	54600	73700

PS, primary sludge; WAS, waste activated sludge; RFW, restaurant food waste; G, glycerol; DFW, digested food waste.

2.2. Experimental design

Batch fermentation experiments were conducted in serum bottles with a total volume of 150 ml and working volume of 100 ml. The ratio of substrate to microorganism (F/M) was adjusted to ≈ 2 g COD/g VS in all reactors (Silva et al., 2013). Prior to the experiments, the initial pH values were adjusted to 5 and 10 by addition of 2 M solutions of HCl and $Mg(OH)_2$, respectively. These two initial pH values were chosen as representative of acidic and alkaline conditions with high VFA production efficiency based on literature (Atasoy et al., 2019; Dahiya et al., 2015).

Bottles were sparged with nitrogen gas for 10 min to ensure anaerobic conditions. Afterwards, the bottles were capped with rubber stoppers, sealed with paraffin film and incubated at 35 °C with orbital shaking of 125 rpm. In total, 126 bottles (21 for each experiment) were set up. Each experiment prolonged for 30 days and samples were analyzed with 5-day intervals. Each experiment was carried out in triplicates. Therefore, on each respective retention time, 3 bottles were opened for analysis.

2.3. Analytical methods

Total solids (TS) and volatile solids (VS) were measured according to Standard Methods (APHA, AWWA, WEF., 2012). Aliquots of the fermentation broth of each reactor was taken and centrifuged at 11000 rpm for 3 min. The supernatant was filtered with 0.45 and 0.2 μm polypropylene filters in order to determine the soluble chemical oxygen demand (sCOD) and VFA composition, respectively. The sCOD was measured using COD cuvette tests (LCK 514 Hach Lange, Germany). The pH was measured by the pH-meter (Mettler Toledo FiveEasyTM pH bench meter, FE20).

The composition of the VFAs were determined using a 9000 Intuvo gas chromatography (Agilent, USA) equipped with CP-Sil 5 CB column (25mx0,32mmx5μm, Agilent) filled with GDX-103 as the stationary phase and a flame ionization detector. Helium was used as the carrier gas. Volatile Free Acid Mix (SupelCo, USA) was used as a VFA standard. VFA concentrations are expressed as the COD equivalent. The COD conversion factor for acetic acid, propionic acid, butyric acid, valeric acid, isovaleric acid and caproic acid was calculated as 1.066, 1.512, 1.816, 2.037, 2.037 and 2.2, respectively (Supplementary documents 1), which can be also found in the literature (Atasoy et al., 2020).

2.4. Microbial community analysis

The structure of the microbial community in the batch reactors were characterized by high-throughput sequencing.

Total genomic DNA was extracted from the samples using NuceloSpin Soil DNA kit (Macherey-Nagel, Germany) according to the manufacturer's instructions.

PCR amplifications of the bacterial and archaeal 16S rRNA genes were performed using MyTaq Red DNA Polymerase (Bioline Reagents Ltd., London, UK) with primers 515F (GTGYCAGCMGCCGCGGTAA)- 806R (GGACTACNVGGGTWTCTAAT) (Caporaso et al., 2011) and Mastercycler Pro thermal cycler (Eppendorf UK Ltd., Stevenage, UK). The amplification conditions were as follows: initial denaturation at 95 °C for 5 min, 35 cycles at 95 °C for 1min, 55 °C for 1 min, 72 °C for 1.5 min, and a final elongation step at 72 °C for 5 min. The PCR products were cleaned using Charge Switch PCR Clean-up kit (Invitrogen, CA, USA), prepared for sequencing according to Caporaso et al. (2012) and sequenced on Illumina MiSeq (300 bp paired-end, Illumina, Inc, San Diego, CA, USA) at the SciLifeLab at KTH-Royal Institute of Technology (Sweden).

2.4.1. Sequence analysis

Merging, quality filtering of the raw sequences and taxonomy assignment were carried out using QIIME2 (Bolyen et al., 2018). Greengenes database was used for the taxonomy assignments for bacteria and archaea at 97% similarly cut-off value. (DeSantis et al., 2006). Sequences are available at the National Center for Biotechnology Read Archive under the project number of PRJNA645049.

2.5. Statistical analysis

The Principal Component Analysis (PCA) was applied to the bacterial diversity data to discriminate the samples according to the operational conditions. Pearson's correlation analysis was conducted to identify the relationship between the bacterial diversity (relative abundance on family level) and VFA composition (acetic, butyric, propionic acids, total VFAs). All analysis was conducted by using IBM SPSS Statistics, Version 26 and PAST 3.

3. Results and discussion

The influence of inoculum, pH and retention time on the VFA productivity and distribution from anaerobic digestion of food waste was investigated. The anaerobic batch reactors were inoculated with three different anaerobic digester sludge operated under acidic (pH 5) and initial alkaline (pH 10) conditions for a period of 30 days.

3.1. Total VFA production

Our results showed that pH, type of inoculum and retention time affected the VFA production efficiency. Fig. 1 shows the total VFAs and their composition obtained from the reactors with the three inocula tested. The highest VFA concentration was 21981 ± 1036 mgCOD/L at day 15 using the inoculum acclimated only to FW (inoculum 3) under initial pH 10. This was followed by 16415 ± 930 mgCOD/L and 11413 ± 408 mgCOD/L by inoculum 2 and 1 at day 10, respectively, under initial pH 10. Initial alkaline pH was more beneficial to the overall performance of the process resulting in higher solubilization of the materials and subsequently higher VFA production compared to initial acidic pH, regardless of the origin of the inoculum.

Under acidic pH, the highest VFAs concentrations was obtained at day 15, which were 12927 ± 1029 mgCOD/L, 12362 ± 860 mgCOD/L and 7036 ± 87 mgCOD/L for inoculum 3, inoculum 2 and inoculum 1, respectively. Using inoculum 3, VFA concentration was more than 1.7 times higher with initial pH 10, compared to the reactors with the same inoculum operated under initial pH 5. The initial alkaline condition can be considered as a pre-treatment step boosting the hydrolysis rate (Garcia-Aguirre et al., 2017). The extracellular polymeric substances (EPS) of sludge contain charged functional groups such as carboxylic groups (Maddela et al., 2018). Under alkaline conditions, these charged groups get ionized and by

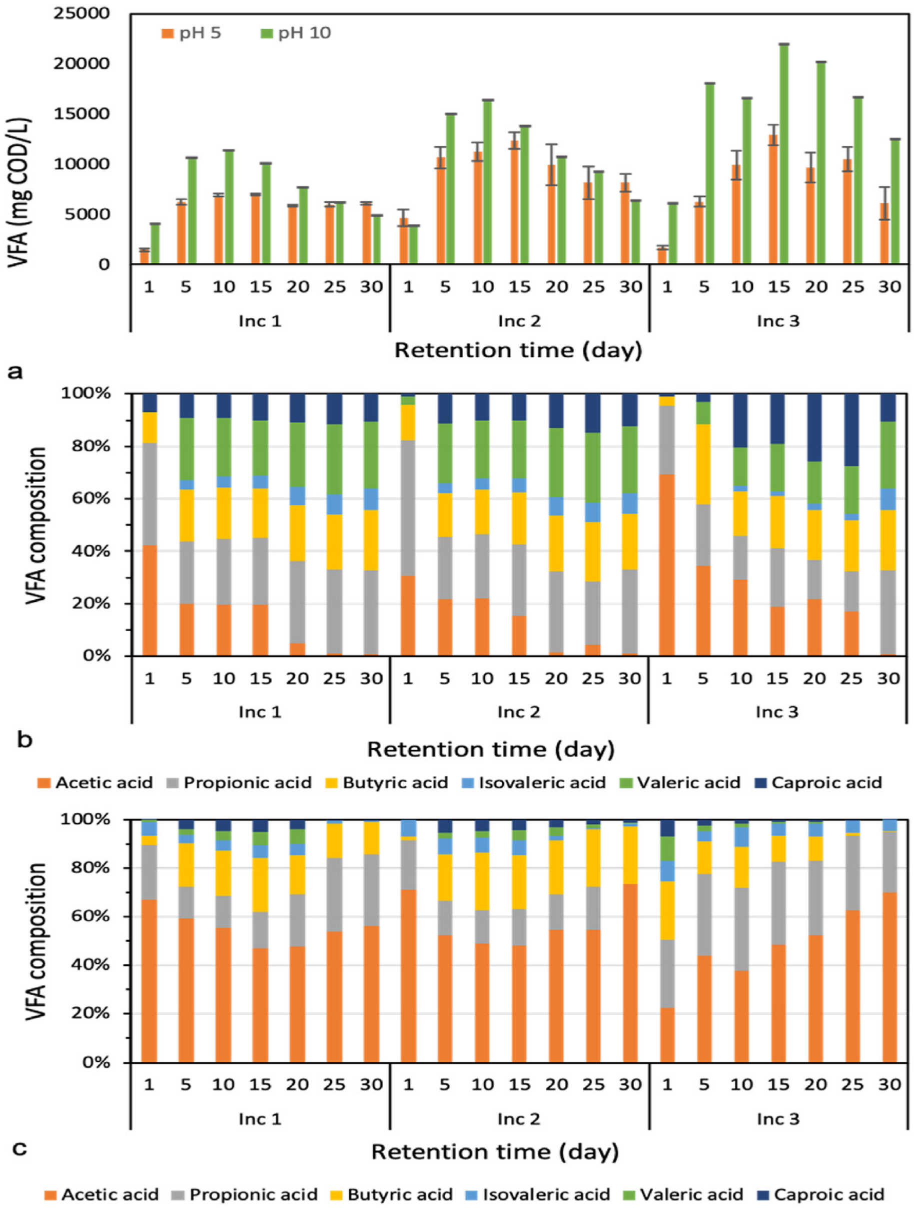

Fig. 1. Total VFA production (a) and their composition respective to each inoculum under pH 5 (b) and pH 10 (c).

promoting the accessibility of the soluble compounds, hydrolysis of the carbohydrates and proteins are improved (Dahiya et al., 2015). This can be the explanation for acquiring higher VFA concentrations in all the reactors with initial pH 10. Similar to us (Zhang et al., 2020), concluded higher solubilization of food waste by increasing the initial pH in AD under mesophilic conditions. Stronger alkaline conditions (pH 10 and higher) circumvents and decreases the growth of methanogens, hence less VFAs are converted into biogas, resulting in higher VFAs accumulations (Jankowska et al., 2017). Higher VFA accumulations under initial alkaline conditions can also be attributed to the higher enrichment of the hydrolytic bacterial species in the reactors such as families *Ruminococcaceae*, *Lachnospirellaceae* and etc, (Section 3.4), which promote decomposition of organic materials by secretion of extracellular hydrolyzing enzymes and subsequent VFA production from organic compounds (Blasco et al., 2020).

In both acidic and alkaline set-ups, the VFA concentrations showed an increasing trend up to day 15 and decreased afterwards. After day 15 the pH values stabilized around neutral conditions (explained in section 3.3) which is the optimal pH range for methanogenesis step (Ren et al., 2018). Moreover, methanogens were found in the microbial community (Supplementary documents 2), which can be the explanation for the decrease in VFA concentrations.

In addition, the VFA yields were also calculated for the retention times with the highest total VFA concentrations. Under initial pH 10, the highest VFA yields were 0,486, 0,393 and 0,378 g COD/gVS for inoculum 2, inoculum 3 and inoculum 1, respectively. In the reactors with initial pH 5, the VFA yields were 0,359, 0,222 and 0,221 for inoculum 2, inoculum 1 and inoculum 3, respectively.

Comparing total VFA production and yields between different studies can be complicated due to the differences in operational parameters, inocula and bacterial structure as well as substrate compositions (Bazyar Lakeh et al., 2019; Shewa et al., 2020). The highest VFA accumulation in our study under pH 5 was 12927 ± 1029 mg COD/L. However, Wang et al. (2014) obtained VFA accumulations up to 34600 mg COD/L from FW fermentation using anerobic activated sludge as inoculum under similar initial pH. The reason for higher VFA concentration can be the difference in the characteristics of the FW as well as the initial COD value in the reactors. Wang et al. (2014) collected the FW from a canteen and it was mostly comprised of meat, vegetables and rice. After removing the bones and other similar particles, their FW slurry was passed through sieves. However, no sorting or sieving of the FW was carried out prior to our experiments, as explained in section 2.1 that the food waste in this study was hygienized in the WWTP and this hygenization can be considered as a pre-treatment. As a result, big plastic particles were observed in some of the reactors. The reason was to eliminate as many operational steps as possible, in order to decrease the operational costs. In line with our results, Dahiya et al. (2015) also obtained the highest VFA productivity (6.3 g/L) from canteen based composite FW at pH 10 compared to 4.2 g/L of VFAs obtained at pH 5. Bermúdez-Penabad et al. (2017) studied the influence of pH on batch anaerobic fermentation of tuna waste. Investigating different initial pH values from 5 to 10, the highest VFA concentration was 28177 mg COD/L at pH 9. Chen et al. (2013) evaluated the co-fermentation of food waste (88%) and dewatered sludge (12%) under different initial pH conditions. The highest sCOD was obtained at pH 11. However, the highest VFA accumulation was at pH 9 (more than 23000 mg COD/L). Authors concluded that stronger alkaline conditions culminate in higher hydrolysis and subsequent VFA production.

We obtained the highest VFA yield of 0,486 g COD/gVS under initial alkaline pH. This is similar to the VFA yields acquired by (Shewa et al., 2020) in the FW fermentation under controlled pH in neutral conditions which acquired yields of 0,429 g COD/gVS. In our results the VFA yields under initial pH 5 were 0,221 and 0,222 g COD/gVS for inoculum 1 and 3, respectively. These are consistent with the yield of 0,255 g COD/gVS obtained by Bazyar Lakeh et al. (2019), from fermentation of source separated FW under initial pH 5. However, using inoculum 2 with initial pH 5, the highest VFA yield was increased to 0,359 g COD/gVS in our study.

3.2. VFA composition

Composition of the VFAs during the fermentation process is of great importance as it stipulates the end-use of the products and their market value. Our results showed that the initial pH changed the composition and distribution of the produced acids. Acetic acid was the main product (38–57% of the total VFAs) in the pH 10 reactors regardless of the inoculum in the first 15 days. In the reactors with pH 5, propionic acid was dominant with the average of 28% and 31% of the total produced VFAs in the first 15 days when inoculum 1 and 2 were used, respectively. However, acetic acid dominated the products in inoculum 3 reactors constituting on average, up to 38% of the total VFAs (Fig. 1). When the ratio of the propionic and acetic acid concentration exceeds 1.4, this is considered as an imbalance and failure of the AD process (Hill et al., 1987; Pullammanappallil et al., 2001). Regardless of the used inocula or initial pH, this threshold was not surpassed in any of the set-ups, until retention time of 20 days.

Higher concentrations of Isovaleric acid was produced in alkaline reactors compared to acidic ones. Under pH 5, the isovaleric concentrations at day 15 were 363 $\pm$ 3, 653 $\pm$ 87 and 230 mg COD/L for inoculum 1, inoculum 2 and inoculum 3, respectively. Whereas, those obtained from pH 10 reactors were 484 $\pm$ 37, 1016 $\pm$ 87 and 1077 $\pm$ 57 mg COD/L for inoculum 1, inoculum 2 and inoculum 3, respectively. Furthermore, higher percentages of valeric and caproic acids were produced under initial pH 5 reactors. In the reactors with initial acidic pH, the decrease in acetic acid concentrations resulted in an increasing trend of caproic acid. From day 1–15, the caproic acid increased from 7 to 10%, 0.78–10% and 0.8–18.89% for inoculum for inoculum 1, inoculum 2 and inoculum 3, respectively. Under pH 5 reactors, the valeric acid production increased from 0 to 20.95%, 3.36–22.2% and 0–18.17% for inoculum 1, inoculum 2 and inoculum 3, respectively.

In general, acetic acid, propionic acid and butyric acid were the three major products accounting for 71–77% and 86–89% of the total produced VFAs until day 15 for reactors with initial pH 5 and initial pH 10, respectively. Thus, mixed-acid fermentation pathway was the dominant metabolic pathway in our experiments. As explained above, the distribution of the acquired metabolites varied among different set-ups, which is a consequence of different pH and inocula (Zhou et al., 2018). The dissociation of the generated organic acids are affected by the operational pH (Hoelzle et al., 2014), hence different products distribution. Acetic acid has a lower market value (400–800 €/ton) compared to butyric acid (1500–1650 €/ton) and propionic acid (2000–2500 €/ton) (Bhatia and Yang, 2017; Cheryan, 2009; Zigová and Šturdík, 2000). However, its current market size (kton/year) is approximately 155 and 37 times higher compared to butyric and propionic acid, respectively (Atasoy et al., 2018). Therefore, these results indicate that initial pH adjustment can be a strategy for aiming higher concentrations of a particular acid; depending on the end use of the products.

Acetic acid was the major product of AD of organic wastes (mixture of the same food waste used in our study with primary sludge) under pH 5, followed by propionic acid in a study by (Owusu-Agyeman et al., 2020). Shifting the pH from 5 to 10, Garcia-Aguirre et al. (2017) obtained higher concentrations of acetic acid. Improved acetic acid production can be attributed to the dominancy of phosphoroclastic pathway under alkaline conditions (Dahiya et al., 2015). Similar to us (Garcia-Aguirre et al., 2017), also attained lower concentrations of isovaleric from different organic wastes under pH 5. The reason can be that isovaleric is mostly the product of protein degradation (Wang et al., 2014). Alkaline conditions enhance the solubilization and degradation of proteins since OH^- ions streamline the breaking of peptide bonds and the formation of free amino acids (Dahiya et al., 2015).

Two-way Anova tests were performed in order to ascertain the significant difference of the VFA production among the three different inocula under each pH. The results indicated a statistically significant difference in the VFA production ($p < 0.005$) under pH 5 and 10, respectively.

3.3. The pH alterations during fermentation

The value of pH plays a prominent role in the bio-production of VFAs as it regulates the activities of different microbes involved in anaerobic digestion (Hobbs et al., 2018). Fig. 2 shows the pH variations over time under both acidic and alkaline conditions. In acidic experiments, the pH was initially adjusted to 5. In the reactors with inoculum 1 and 2, pH values remained within the range of 5 $\pm$ 0.4 in the first 15 days due to acids production. After day 15, a gradual increase in the pH was observed until it reached 5.9 $\pm$ 0.1 at the end of the experimental period, while the inoculum 3 experiments exhibited a different trend and had lower pH fluctuations. The pH of 5 $\pm$ 0.1 was sustained throughout the experimental period.

In the alkaline experiments, the initial pH was adjusted to 10 and decreased to 6.5 after one day in the reactors seeded with inoculum 1 and inoculum 2. The declining trend continued until day 10 where it reached the value of 5.9. Afterwards, pH started to increase until the values of 7.3 $\pm$ 0.05 in day 30. In the experiments seeded with inoculum 3 under alkaline conditions, the pH drop was smaller compared to the other inocula. pH dropped to 7.3 in the first day and continued a decreasing trend until day 10 where it reached

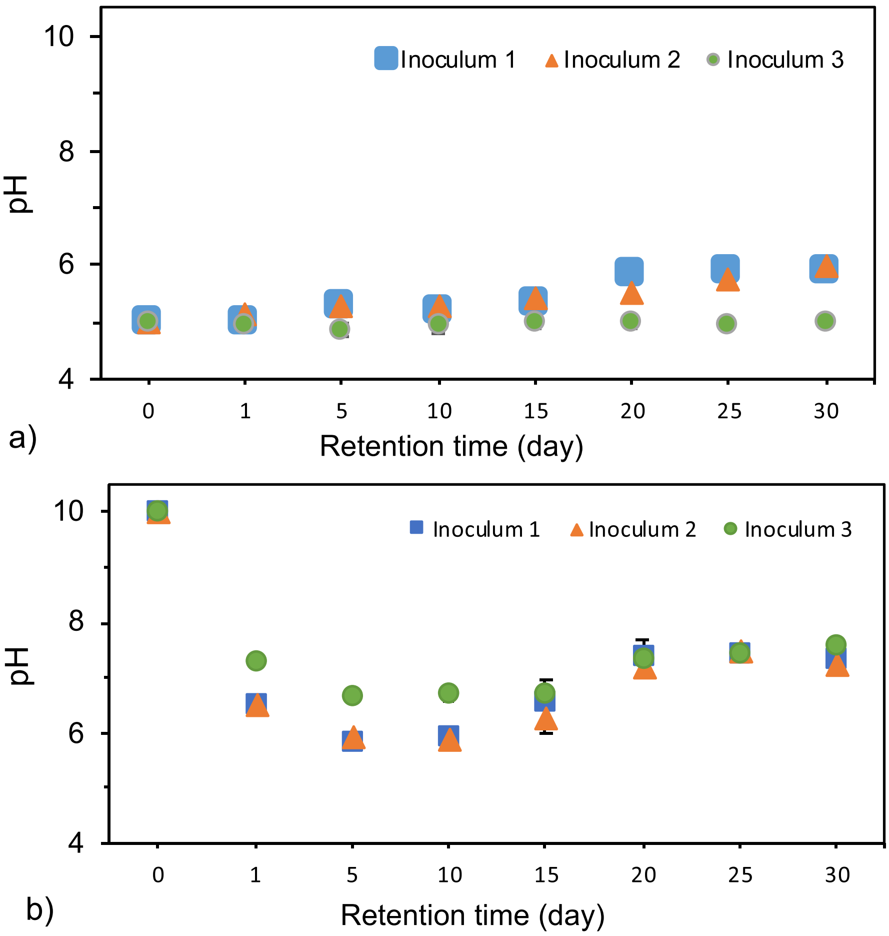

Fig. 2. Variation of pH with respect to retention time under pH 5 (a) and pH 10 (b) reactors.

the value of 6.7 and remained constant in the next five days. Afterwards pH started to increment until it reached the value of 7.6 at the end of the experimental period.

pH not only plays a significant role in the synthesis of VFAs, but also is a crucial factor affecting the composition of the end-products. Similar to our results, Jankowska et al. (2017) concluded that while initial alkaline pH enhances the hydrolysis of organic materials, acidogenesis is higher under neutral conditions. Additionally, Atasoy et al. (2019) illustrated that the dominant VFA can be varied based on the initial pH. In their experiments, while acetic and butyric acids were the dominant products under initial pH 5 fermentations, butyric acid was the major product of the initial alkaline mixed culture fermentation. In another study using cheese processing wastewater under pH 10, the effects of shifting from batch to sequencing batch reactors were examined. While the obtained VFA production yields were similar, the type of dominant acids were altered based on the reactor type (Atasoy et al., 2020). Furthermore, Owusu-Agyeman et al. (2020) demonstrated that the composition of the VFAs obtained from digestion of organic waste in a batch reactor with initial pH 5 differ from that obtained from semi-continuous reactor with pH controlled at 5. Therefore, regulating the operational pH can be deemed as a way of maintaining a high VFA production efficiency as well as aiming for a specific acid type, particularly; in long term and continuous operations. However, it must be noted that the optimum pH for generation of a specific acid, relies highly on the waste stream used as the substrate (Lee et al., 2014).

3.4. Bacterial community analysis

The bacterial community structure was analyzed in order to evaluate its effect on the VFA production efficiency as well as the VFA composition. In our experiments, the dominant taxa at the phylum level were *Firmicutes*, *Chloroflexi* and *Bacteroidetes*. At the family level (Figs. 3 and 4), the dominant taxa differed under acidic and alkaline pH conditions, which was also supported by PCA. The PCA results demonstrated robust clustering of the family members in the reactors under both pH conditions, particularly until day 15 (Fig. 5). As mentioned before, the VFA concentration also decreased after day 15.

The bacterial composition at pH 5 was comprised of *Anaerolineaceae* 32 ± 12%, *Veillonellaceae* 22 ± 7%, *Clostridiaceae* 9 ± 3%, *Lactobacillaceae* (16 ± 11), *unidentified Bacteroidales* 14 ± 6% and *Ruminococcaceae* 5 ± 4% as the dominant family members., while in reactors at pH 10, *Anaerolineaceae* 29 ± 5%, *Clostridiaceae* 26 ± 11%, *Ruminococcaceae* 15 ± 9%, *Tissierellaceae* 11 ± 8% and *Lachnospirellaceae* 4 ± 1% were the dominant family members. One of the differences in the bacterial communities was the relative abundances of *Clostridiaceae* and *Ruminococcaceae*, which increased more than three times by shifting from initial pH 5 to initial pH 10. Similar to our results, the growth of *Clostridiaceae* was increased with initial alkaline conditions in mixed microbial fermentations using different inocula in a study by (Atasoy et al., 2019). *Clostridiaceae* are a proteolytic bacteria (Ramsay and Pullammanappallil, 2001), which belong to the *Firmicutes* phylum

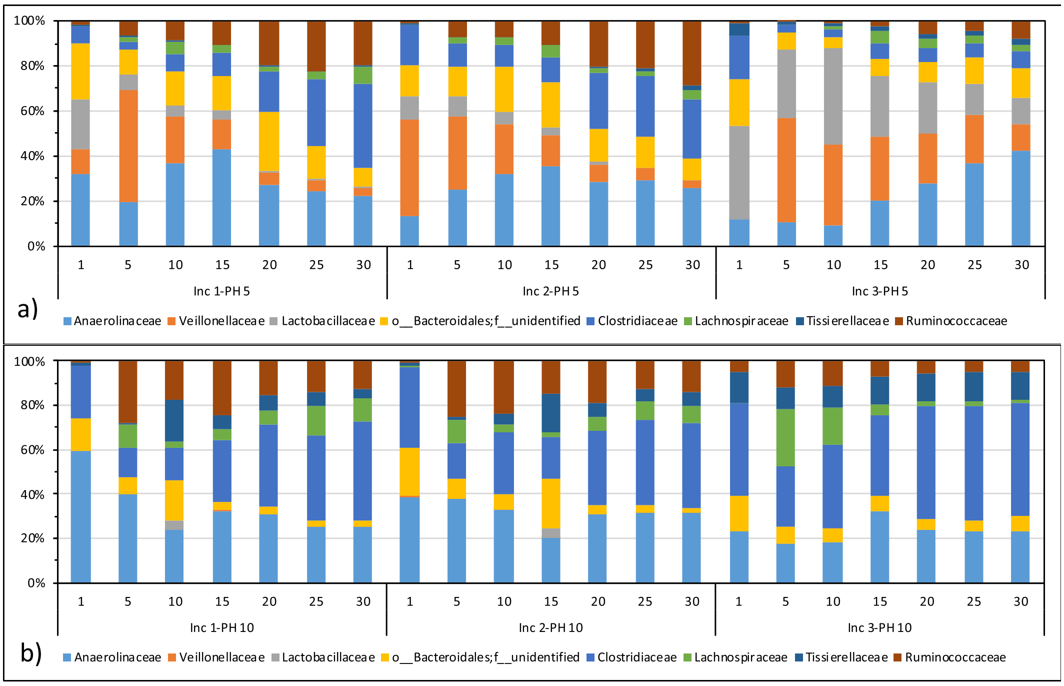

Fig. 3. Relative abundance of the bacterial community in family level a) pH 5 reactors b) pH 10 reactors.

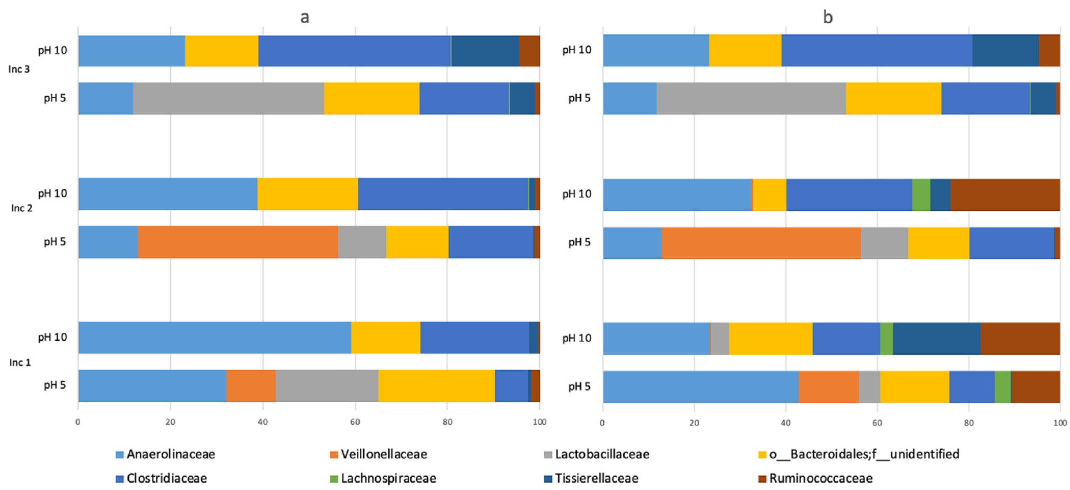

Fig. 4. Changes of bacterial community on family level. Column (a) on day 1 and column (b) on the day with highest VFA accumulation.

and have been associated with the production of butyric acid from hydrolysis of polysaccharides (Fu et al., 2015) as well as production of acetic acid, butanol, acetone, and ethanol (Rosenberg et al., 2014; Wiegel et al., 2006). *Clostridiaceae* area one of the most prevalent family members of anaerobic digestion (Liliana Pampillón-González et al., 2016). A positive correlation (0.488) between *Clostridiaceae* and acetic acid production was shown in all sets. The results of the correlation analysis verify the family *Clostridiaceae* is responsible for acetic acid production in both pH conditions of this study.

Shifting the initial pH from 5 to initial pH of 10, in the retention times with the highest VFA production, the relative abundance of *Ruminococcaceae* increased from 10.7 to 17.3%, 10.3–24% and 2.3–7% for inoculum 1, inoculum 2 and inoculum 3, respectively. *Ruminococcaceae*, another member of the *Firmicutes* phylum, are reported to generate propionic and acetic acid under the pH ranging between 6 and 8 (Feng et al., 2009). However, the results of the correlation analysis indicated a negative relation between *Ruminococcaceae* and acetic acid production in pH 5 reactors. On the other hand, butyric acid concentrations were positively correlated (0.645) with *Ruminococcaceae* (P value < 0.01) in the alkaline reactors (Fig. 7). A syntrophic relationship was observed between the family *Ruminococcaceae* and *Veillonellaceae* (0.876) under pH 10 ($p < 0.01$). Furthermore, both families positively correlated with butyric and caproic acid production. *Veillonellaceae* which also belong to the *Firmicutes* phylum, are responsible for lactate fermentation, hydrolysis and acidification (Slezak et al., 2017). On another note, the relative abundance of the family *Veillonellaceae* was higher in the reactors with initial pH 5. Acetic acid production was positively correlated (0.669) with *Veillonellaceae* in our acidic reactors (P value < 0.01) (Fig. 6).

Anaerolineaceae, from the Chloroflexi phylum, accounted for

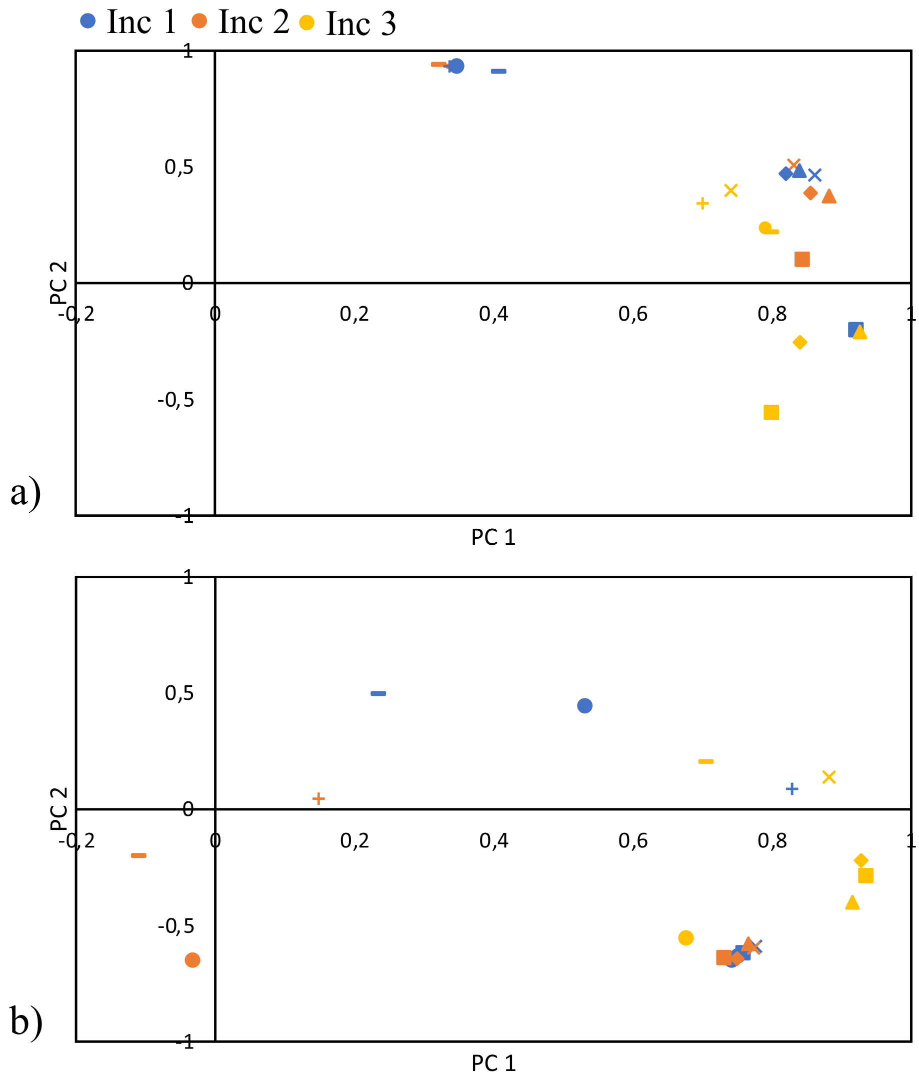

Fig. 5. PCA analysis of the bacterial community on family level: a) pH 5 1 b) pH 10. The inoculum 1, 2 and 3 reactors are shown in blue, orange and yellow, respectively. Each retention time is depicted as: (■) day 1 (◆) day 5 (▲) day 10 (×) day 15 (–) day 20 (●) day 25 (+) day 30. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

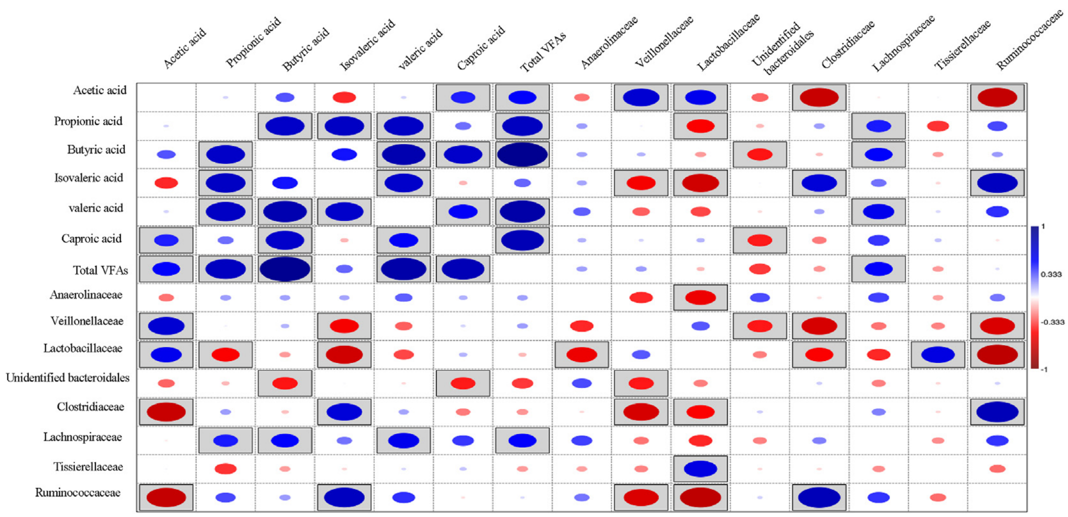

Fig. 6. Correlation analysis between relative abundance of bacterial family level with each acid type in reactors with initial pH 5.

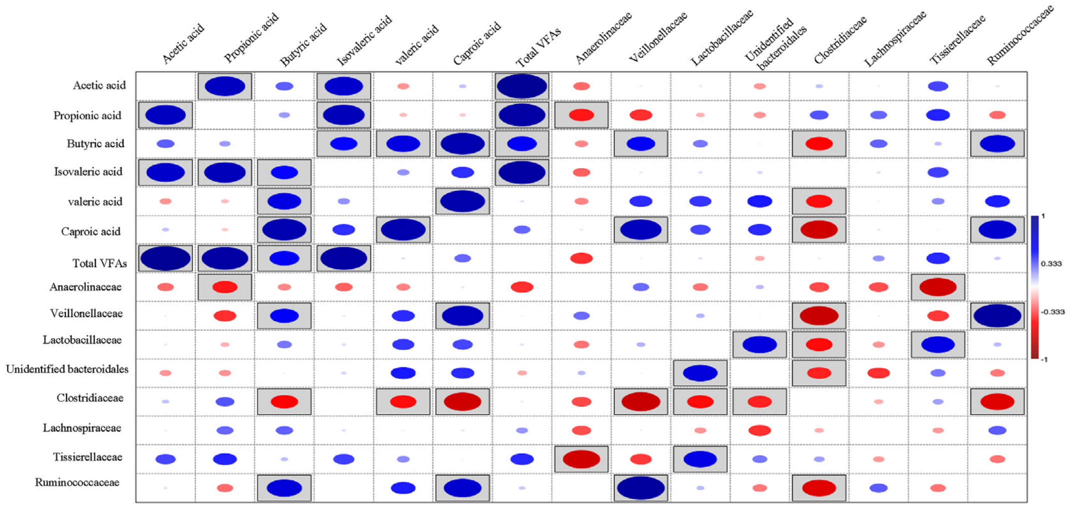

Fig. 7. Correlation analysis between relative abundance of bacterial family level with each acid type in reactors with initial pH 10.

32 ± 12% and 29 ± 5% of the dominant families in acidic and alkaline reactors, respectively. They are known as a non-hydrolytic acidogens which generate low molecular weights products such as acetate and H_2 from carbohydrates (Liang et al., 2015; McIlroy et al., 2017). Members of the *Anaerolineaceae* family are associated with the formation and maintenance of granular structure of sludge hence increasing the settleability in upflow anerobic sludge blanket reactors (Yamada and Sekiguchi, 2009; Zhu et al., 2017).

Lactobacillaceae, which are the main contributors of lactic, acetic and propionic acid generation (Oliveira et al., 2014; Tannock, 2004; Yun and Cho, 2016), were only present in the pH 5 reactors. The reason can be that members of the *Lactobacillaceae* family are known to have a high tolerance to acidic environments (Sträuber et al., 2012). In our study, while acetic acid was positively correlated (0.564) with *Lactobacillaceae* ($p < 0.01$), this family demonstrated a negative correlation (−0.509) with propionic acid concentrations (P value < 0.05) (Fig. 6).

3.5. Future perspectives of VFA production from waste streams

Bio-based production of VFAs has been recognized as a sustainable approach for recovery of value-added products from waste streams, in the recent years. Yet, 1) enhancing the production efficiency and 2) separation and recovery of the end-products from the fermentation broth are deemed as the main hurdles of large scale applications (Atasoy et al., 2018; Strazzera et al., 2018). Bioaugmentation of the microbial community has been proposed as a solution to not only improve the total VFA production; but also to aim for a specific acid type. Atasoy and Cetecioglu (2020), bioaugmented *Clostridium butyricum* in dairy wastewater. They acquired 11 times and 3.5 times higher butyric acid and total VFA production, respectively. These promising results propel the manipulation of the functional bacterial community in conjunction with other operational parameters for future large-scale applications.

The second challenge of commercial VFA production is associated with the downstream processing and its economical burdens. Woo and Kim (2019), have recently proposed a combination of extraction and distillation strategy for 99% VFA recovery with 99.5% purity. Their operational cost for separation is estimated as 0.53 $/kg VFA, making the process profitable for up-scaling applications with 2.70 $ as the price of 1 kg of mixture of produced acids. Additionally, in-situ separation of VFAs in the reactors is another solution for facilitating the downstream processing. Using a side stream membrane bioreactors (MBR), Lukitawesa et al. (2020) was able to maintain high reactor production yields with high organic loading rates (4 g vs/L.d). In semi-continuous operation, their MBRs did not encounter clogging, nor fouling which suggest promising overviews for long term applications of this technology.

Moreover, integration of VFA production in WWTPs and their further conversion into other value-added products have gained considerable attention in pilot scale applications. One example is the Carbonera WWTP in Italy, which has evaluated the biological nutrient removal and polyhydroxyalkanoates production from waste-derived VFAs (Conca et al., 2020). Their results of 80% ammonia removal as well as high PHA yield of 0.58−0.61 $gCOD_{PHA}/gCOD_{VFA,}$ is promising for potential success of future large scale VFA production and their utilization as renewable carbon sources.

4. Conclusions

Food waste is a suitable raw material for VFA production through anaerobic digestion. Inoculum, pH and retention time are three of the parameters influencing the VFA production process. Initial alkaline conditions resulted in substantially higher VFA accumulations compared to acidic conditions. Furthermore, initial pH value altered the composition of the obtained VFAs. The inoculum acclimated to food waste resulted in highest VFA accumulation with initial alkaline conditions. For the long-term operations, pH regulation and bioaugmentation of particular species can be plausible approaches for increasing the VFA production as well as generation of a specific acid type.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.129981.

References

APHA, AWWA, WEF, 2012. Standard Methods for examination of water and wastewater. APHA, AWWA, WEF. "Standard Methods Exam. water wastewater. https://doi.org/10.5209/rev_ANHM.2012.v5.n2.40440.
Atasoy, M., Cetecioglu, Z., 2020. Butyric acid dominant volatile fatty acids production: bio-Augmentation of mixed culture fermentation by Clostridium butyricum. J. Environ. Chem. Eng. https://doi.org/10.1016/j.jece.2020.104496.
Atasoy, M., Eyice, O., Cetecioglu, Z., 2020. A comprehensive study of volatile fatty acids production from batch reactor to anaerobic sequencing batch reactor by using cheese processing wastewater. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2020.123529.
Atasoy, M., Eyice, O., Schnürer, A., Cetecioglu, Z., 2019. Volatile fatty acids production via mixed culture fermentation: revealing the link between pH, inoculum type and bacterial composition. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2019.121889.
Atasoy, M., Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z., 2018. Bio-based volatile fatty acid production and recovery from waste streams: current status and future challenges. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2018.07.042.
Bazyar Lakeh, A.A., Azizi, A., Hosseini Koupaie, E., Bekmuradov, V., Hafez, H., Elbeshbishy, E., 2019. A comprehensive study for characteristics, acidogenic fermentation, and anaerobic digestion of source separated organics. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2019.04.223.
Bermúdez-Penabad, N., Kennes, C., Veiga, M.C., 2017. Anaerobic digestion of tuna waste for the production of volatile fatty acids. Waste Manag. https://doi.org/10.1016/j.wasman.2017.06.010.
Bhatia, S.K., Yang, Y.H., 2017. Microbial production of volatile fatty acids: current status and future perspectives. Rev. Environ. Sci. Biotechnol. https://doi.org/10.1007/s11157-017-9431-4.
Blasco, L., Kahala, M., Tampio, E., Vainio, M., Ervasti, S., Rasi, S., 2020. Effect of inoculum pretreatment on the composition of microbial communities in anaerobic digesters producing volatile fatty acids. Microorganisms. https://doi.org/10.3390/microorganisms8040581.
Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E., Da Silva, R., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson II, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2018. QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. PeerJ Prepr 6. https://doi.org/10.7287/peerj.preprints.27295v2 e27295v2.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. Unit. States Am. 108, 4516–4522. https://doi.org/10.1073/pnas.1000080107.
Capson-Tojo, G., Rouez, M., Crest, M., Steyer, J.P., Delgenès, J.P., Escudié, R., 2016. Food waste valorization via anaerobic processes: a review. Rev. Environ. Sci. Biotechnol. https://doi.org/10.1007/s11157-016-9405-y.
Chen, H., Meng, H., Nie, Z., Zhang, M., 2013. Polyhydroxyalkanoate production from fermented volatile fatty acids: effect of pH and feeding regimes. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2012.10.121.
Cheryan, M., 2009. Acetic acid production. In: Encyclopedia of Microbiology. https://doi.org/10.1016/B978-012373944-5.00128-0.
Commision Expert Group on Bio-Based Products, 2017.
Conca, V., da Ros, C., Valentino, F., Eusebi, A.L., Frison, N., Fatone, F., 2020. Long-term validation of polyhydroxyalkanoates production potential from the sidestream of municipal wastewater treatment plant at pilot scale. Chem. Eng. J. https://doi.org/10.1016/j.cej.2020.124627.
Dahiya, S., Sarkar, O., Swamy, Y.V., Venkata Mohan, S., 2015. Acidogenic fermentation of food waste for volatile fatty acid production with co-generation of biohydrogen. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2015.01.007.
De Gioannis, G., Muntoni, A., Polettini, A., Pomi, R., 2013. A review of dark fermentative hydrogen production from biodegradable municipal waste fractions. Waste Manag. https://doi.org/10.1016/j.wasman.2013.02.019.
DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072. https://doi.org/10.1128/AEM.03006-05.
European Commission, 2019. The European green deal, COM(2019) 640 final. https://doi.org/10.2307/j.ctvd1c6zh.7.
Feng, L., Chen, Y., Zheng, X., 2009. Enhancement of waste activated sludge protein conversion and volatile fatty acids accumulation during waste activated sludge anaerobic fermentation by carbohydrate substrate addition: the effect of pH. Environ. Sci. Technol. https://doi.org/10.1021/es8037142.
Fu, S.F., He, S., Shi, X.S., Katukuri, N.R., Dai, M., Guo, R.B., 2015. The chemical properties and microbial community characterization of the thermophilic microaerobic pretreatment process. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2015.09.029.
Garcia-Aguirre, J., Aymerich, E., González-Mtnez de Goñi, J., Esteban-Gutiérrez, M., 2017. Selective VFA production potential from organic waste streams: assessing temperature and pH influence. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.07.187.
Hill, D.T., Cobb, S.A., Bolte, J.P., 1987. Using volatile fatty acid relationships to predict anaerobic digester failure. Trans. Am. Soc. Agric. Eng. https://doi.org/10.13031/2013.31978.
Hobbs, S.R., Landis, A.E., Rittmann, B.E., Young, M.N., Parameswaran, P., 2018. Enhancing anaerobic digestion of food waste through biochemical methane potential assays at different substrate: inoculum ratios. Waste Manag. https://doi.org/10.1016/j.wasman.2017.06.029.
Hoelzle, R.D., Virdis, B., Batstone, D.J., 2014. Regulation mechanisms in mixed and pure culture microbial fermentation. Biotechnol. Bioeng. https://doi.org/10.1002/bit.25321.
Jankowska, E., Chwialkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2017. Volatile fatty acids production during mixed culture fermentation – the impact of substrate complexity and pH. Chem. Eng. J. https://doi.org/10.1016/j.cej.2017.06.021.
Khatami, K., Perez-Zabaleta, M., Owusu-Agyeman, I., Cetecioglu, Z., 2020. Waste to bioplastics: how close are we to sustainable polyhydroxyalkanoates production? Waste Manag. https://doi.org/10.1016/j.wasman.2020.10.008.
Kim, M., Gomec, C.Y., Ahn, Y., Speece, R.E., 2003. Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion. Environ. Technol. https://doi.org/10.1080/09593330309385659.
Kim, N.-J., Lim, S.-J., Chang, H.N., 2018. Volatile fatty acid platform: concept and application. In: Emerging Areas in Bioengineering. https://doi.org/10.1002/9783527803293.ch10.
Kleerebezem, R., Joosse, B., Rozendal, R., Van Loosdrecht, M.C.M., 2015. Anaerobic digestion without biogas? Rev. Environ. Sci. Biotechnol. https://doi.org/10.1007/s11157-015-9374-6.
Komemoto, K., Lim, Y.G., Nagao, N., Onoue, Y., Niwa, C., Toda, T., 2009. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. Waste Manag. https://doi.org/10.1016/j.wasman.2009.07.011.
Lee, W.S., Chua, A.S.M., Yeoh, H.K., Ngoh, G.C., 2014. A review of the production and applications of waste-derived volatile fatty acids. Chem. Eng. J. 235, 83–99. https://doi.org/10.1016/j.cej.2013.09.002.
Li, Z., Chen, Z., Ye, H., Wang, Y., Luo, W., Chang, J.S., Li, Q., He, N., 2018. Anaerobic co-digestion of sewage sludge and food waste for hydrogen and VFA production with microbial community analysis. Waste Manag. https://doi.org/10.1016/j.wasman.2018.06.046.
Liang, B., Wang, L.Y., Mbadinga, S.M., Liu, J.F., Yang, S.Z., Gu, J.D., Mu, B.Z., 2015. Anaerolineaceae and Methanosaeta turned to be the dominant microorganisms in alkanes-dependent methanogenic culture after long-term of incubation. Amb. Express. https://doi.org/10.1186/s13568-015-0117-4.
Liu, G., Liu, X., Li, Y., He, Y., Zhang, R., 2011. Influence of pH adjustment and inoculum on anaerobic digestion of kitchen waste for biogas producing. J. Biobased Mater. Bioenergy. https://doi.org/10.1166/jbmb.2011.1161.
Lukitawesa, Eryildiz, B., Mahboubi, A., Millati, R., Taherzadeh, M.J., 2020. Semi-continuous production of volatile fatty acids from citrus waste using membrane

bioreactors. Innovat. Food Sci. Emerg. Technol. https://doi.org/10.1016/j.ifset.2020.102545.
Maddela, N.R., Zhou, Z., Yu, Z., Zhao, S., Meng, F., 2018. Functional determinants of extracellular polymeric substances in membrane biofouling: experimental evidence from pure-cultured sludge bacteria. Appl. Environ. Microbiol. https://doi.org/10.1128/AEM.00756-18.
McIlroy, S.J., Kirkegaard, R.H., Dueholm, M.S., Fernando, E., Karst, S.M., Albertsen, M., Nielsen, P.H., 2017. Culture-independent analyses reveal novel anaerolineaceae as abundant primary fermenters in anaerobic digesters treating waste activated sludge. Front. Microbiol. https://doi.org/10.3389/fmicb.2017.01134.
Oliveira, P.M., Zannini, E., Arendt, E.K., 2014. Cereal fungal infection, mycotoxins, and lactic acid bacteria mediated bioprotection: from crop farming to cereal products. Food Microbiol. https://doi.org/10.1016/j.fm.2013.06.003.
Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z., 2020. Production of volatile fatty acids through co-digestion of sewage sludge and external organic waste: effect of substrate proportions and long-term operation. Waste Manag. https://doi.org/10.1016/j.wasman.2020.05.027.
Pullammanappallil, P.C., Chynoweth, D.P., Lyberatos, G., Svoronos, S.A., 2001. Stable performance of anaerobic digestion in the presence of a high concentration of propionic acid. Bioresour. Technol. https://doi.org/10.1016/S0960-8524(00)00187-5.
Ramsay, I.R., Pullammanappallil, P.C., 2001. Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. Biodegradation. https://doi.org/10.1023/A:1013116728817.
Rebecchi, S., Pinelli, D., Bertin, L., Zama, F., Fava, F., Frascari, D., 2016. Volatile fatty acids recovery from the effluent of an acidogenic digestion process fed with grape pomace by adsorption on ion exchange resins. Chem. Eng. J. https://doi.org/10.1016/j.cej.2016.07.101.
Ren, Y., Yu, M., Wu, C., Wang, Q., Gao, M., Huang, Q., Liu, Y., 2018. A comprehensive review on food waste anaerobic digestion: research updates and tendencies. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.09.109.
Rosenberg, E., Delong, E.F., Thompson, F., 2014. The Prokaryotes, Firmicutes and Tenericutes, fourth ed. Springer Heidelberg, New York Dordrecht London, London. https://doi.org/10.1007/978-3-642-30120-9.
Scherhaufer, S., Moates, G., Hartikainen, H., Waldron, K., Obersteiner, G., 2018. Environmental impacts of food waste in Europe. Waste Manag. https://doi.org/10.1016/j.wasman.2018.04.038.
Shewa, W.A., Hussain, A., Chandra, R., Lee, J., Saha, S., Lee, H.S., 2020. Valorization of food waste and economical treatment: effect of inoculation methods. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2020.121170.
Silva, F.C., Serafim, L.S., Nadais, H., Arroja, L., Capela, I., 2013. Acidogenic fermentation towards valorisation of organic waste streams into volatile fatty acids. Chem. Biochem. Eng. Q.
Slezak, R., Grzelak, J., Krzystek, L., Ledakowicz, S., 2017. The effect of initial organic load of the kitchen waste on the production of VFA and H2 in dark fermentation. Waste Manag. 68, 610−617.
Spekreijse, J., Lammens, T., Parisi, C., Ronzon, T., Vis, M., 2019. Insights into the European Market of Bio-Based Chemicals. Analysis Based on Ten Key Product Categories. https://doi.org/10.2760/673071. Luxembourg.
Sträuber, H., Schröder, M., Kleinsteuber, S., 2012. Metabolic and microbial community dynamics during the hydrolytic and acidogenic fermentation in a leach-bed process. Energy. Sustain. Soc. https://doi.org/10.1186/2192-0567-2-13.
Strazzera, G., Battista, F., Garcia, N.H., Frison, N., Bolzonella, D., 2018. Volatile fatty acids production from food wastes for biorefinery platforms: a review. J. Environ. Manag. https://doi.org/10.1016/j.jenvman.2018.08.039.
Tannock, G.W., 2004. Minireviews A special fondness for lactobacilli. Appl. Environ. Microbiol. https://doi.org/10.1128/AEM.70.6.3189.
Trad, Z., Akimbomi, J., Vial, C., Larroche, C., Taherzadeh, M.J., Fontaine, J.P., 2015. Development of a submerged anaerobic membrane bioreactor for concurrent extraction of volatile fatty acids and biohydrogen production. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2015.07.095.
Wang, K., Yin, J., Shen, D., Li, N., 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2014.03.088.
Wiegel, J., Tanner, R., Rainey, F.A., 2006. An introduction to the family Clostridiaceae. In: The Prokaryotes. https://doi.org/10.1007/0-387-30744-3_20.
Woo, H.C., Kim, Y.H., 2019. Eco-efficient recovery of bio-based volatile C2-6 fatty acids. Biotechnol. Biofuels. https://doi.org/10.1186/s13068-019-1433-8.
Xu, F., Li, Yangyang, Ge, X., Yang, L., Li, Yebo, 2018. Anaerobic digestion of food waste − challenges and opportunities. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.09.020.
Yamada, T., Sekiguchi, Y., 2009. Cultivation of uncultured Chloroflexi subphyla: significance and ecophysiology of formerly uncultured Chloroflexi “subphylum i” with natural and biotechnological relevance. Microb. Environ. https://doi.org/10.1264/jsme2.ME09151S.
Ye, M., Liu, J., Ma, C., Li, Y.Y., Zou, L., Qian, G., Xu, Z.P., 2018. Improving the stability and efficiency of anaerobic digestion of food waste using additives: a critical review. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2018.04.244.
Yun, J., Cho, K.S., 2016. Effects of organic loading rate on hydrogen and volatile fatty acid production and microbial community during acidogenic hydrogenesis in a continuous stirred tank reactor using molasses wastewater. J. Appl. Microbiol. https://doi.org/10.1111/jam.13316.
Zacharof, M.P., Lovitt, R.W., 2013. Complex effluent streams as a potential source of volatile fatty acids. Waste and Biomass Valorization. https://doi.org/10.1007/s12649-013-9202-6.
Zhang, C., Su, H., Baeyens, J., Tan, T., 2014. Reviewing the anaerobic digestion of food waste for biogas production. Renew. Sustain. Energy Rev. https://doi.org/10.1016/j.rser.2014.05.038.
Zhang, L., Loh, K.C., Dai, Y., Tong, Y.W., 2020. Acidogenic fermentation of food waste for production of volatile fatty acids: bacterial community analysis and semi-continuous operation. Waste Manag. https://doi.org/10.1016/j.wasman.2020.04.052.
Zhou, M., Yan, B., Wong, J.W.C., Zhang, Y., 2018. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: a mini-review focusing on acidogenic metabolic pathways. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.06.121.
Zhu, X., Kougias, P.G., Treu, L., Campanaro, S., Angelidaki, I., 2017. Microbial community changes in methanogenic granules during the transition from mesophilic to thermophilic conditions. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-016-8028-0.
Zigová, J., Šturdík, E., 2000. Advances in biotechnological production of butyric acid. J. Ind. Microbiol. Biotechnol. https://doi.org/10.1038/sj.jim.2900795.